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Efficacy of vitamins B9, C, and E on fat graft viability

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ABSTRACT

Aims: This study aims to investigate the potential of folic acid (vitamin B9), ascorbic acid (vitamin C), and α-tocopherol (vitamin E) in enhancing the survival of fat grafts.

Methods: We divided the dorsal regions of ten Sprague-Dawley rats into four quadrants, serving as recipient sites for inguinal fat grafts. Fat grafts were placed in these sites after incubation, with different sites receiving 0.9% NaCl (left cranial), vitamin B9 (right cranial), vitamin C (left caudal), and vitamin E (right caudal). After three months, we harvested the fat grafts and evaluated their viability using perilipin immunohistochemistry.

Results: Folic acid, vitamin C, and vitamin E all significantly improved fat graft survival rates when compared with control ($p<0.001$ for each).

Conclusion: Folic acid (vitamin B9) promotes angiogenesis and collagen synthesis, vitamin C's antioxidant properties support collagen synthesis, and vitamin E's potent antioxidant capacity protects grafted adipose tissue from oxidative stress and inflammation, facilitating graft vascularization and integration. These findings suggest that these readily available and cost-effective vitamins, B9, C, and E, enhance fat graft survival immunohistochemically. The improved graft viability presented here could inspire further clinical studies and applications.

Keywords: Adipogenesis, ascorbic acid, fat graft survival, folic acid, vitamin C, vitamin E

INTRODUCTION

The realm of plastic and reconstructive surgery has witnessed significant transformation with the emergence of fat grafting, a technique renowned for its ability to restore tissue volume and shape, achieving natural-looking results. Nevertheless, challenges persist in maintaining consistent and dependable fat graft survival, driving ongoing efforts to refine methods and introduce complementary strategies.[1] This investigation focuses on the intriguing roles of vitamin B9 (folic acid), vitamin C (ascorbic acid), and vitamin E (α-tocopherol) in influencing fat graft survival outcomes. The utilization of these vitamins, each celebrated for its unique biological properties, offers a new avenue to address the longstanding issue of graft retention variability.

Folic acid, an essential B vitamin vital for cell division and tissue development, has garnered attention for its potential to stimulate angiogenesis and collagen synthesis. These mechanisms suggest a capacity to facilitate graft integration and vascularization, factors critical for graft survival.[2] Vitamin C, known for its antioxidant potential and its role as a cofactor in collagen synthesis, presents an enticing prospect for enhancing fat graft survival. By mitigating oxidative stress and supporting tissue repair mechanisms, vitamin C could contribute to graft integrity and function preservation.[3] Similarly, vitamin E, another potent antioxidant, may offer protection against oxidative damage and inflammation, potentially safeguarding grafted adipose tissue from early resorption.[4] With these promising attributes, this study delves into the existing literature regarding the effects of folic acid, ascorbic acid, and α-tocopherol on fat graft survival, aiming to bridge the gap between theoretical advantages and practical clinical application.

In summary, the integration of vitamin B9, vitamin C, and vitamin E into fat grafting procedures opens up new horizons for enhancing graft survival and overall surgical outcomes.

METHODS

The study was carried out with the permission of Hatay Mustafa Kemal University Animal Experimental Researches Ethics Committee (Date: 21.09.2020, Decision No: 2020/06-6). All relevant institutional and national guidelines for animal care and use were strictly adhered to.

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Surgical Procedure

We divided the dorsal regions of ten male Sprague-Dawley rats, aged between 8-9 weeks and weighing 300±10 grams, into four separate quadrants: left cranial, right cranial, left caudal, and right cranial (Figure 1a). These quadrants served as recipient sites for fat grafts (serum, vitamin B9, vitamin C, and vitamin E groups, respectively).[5] Under general anesthesia, with surgical antibiotic prophylaxis and local antisepsis, inguinal fat was harvested, and approximately four pieces of 0.2-gram fat grafts were obtained. During the surgery, fat grafts, harvested in a sterile environment, were immersed in separate 5 ml solutions, sequentially containing 0.9% sodium chloride, 20 mg/2 ml Vitamin B9 (Folsaure forte-Hevert 20 mg/2 ml Ampoule, Hevert-Arzneimittel GmbH & Co. KG, Nussbaum, Germany), 500 mg/5 ml Vitamin C (Redox-C® Ampul 500 mg/5 ml, Bayer Türk Kimya San. Ltd. Şti., Istanbul, Türkiye), and 100 mg/2 ml Vitamin E (Evicap 100 mg/2 ml Ampul, Koçak Farma İlaç ve Kimya San. A.Ş., Istanbul, Türkiye) (Figure 1b) at 37 degrees Celsius to mimic the body temperature of male Sprague-Dawley rats. Four one-centimeter incisions were made on the back regions, and fat grafts were placed beneath the subcutaneous tissue overlying the muscle fascia (Figure 1c). The incisions in the donor and recipient areas were closed with non-absorbable sutures. The rats were housed individually in cages maintained at standard room temperature and were provided with ad libitum food.

After a three-month period, the rats were euthanized, and the fat grafts, along with the surrounding transition area, were excised (Figure 1d) and evaluated immunohistochemically.

Immunohistochemical Evaluation

Pathological examination service was procured from Merter Medical Company, with the pathologists employed by the company waiving their right to publish. The samples underwent standard processing and were embedded in paraffin blocks. Longitudinal slices of 5 µm thickness were cut from the widest areas of the samples. After routine processing and immunohistochemical staining, the slices were stained with perilipin (PP) to assess adipocyte viability rate (Figure 2). Quantitative results were obtained using Fiji software (ImageJ version 2.1.0, National Institutes of Health, Bethesda, MD, USA), and the data were presented as optic density ± standard deviation.[6]

Statistical Analysis

We performed a power analysis using G*Power 3.1 to determine the required sample size for a one-way analysis of variance. The analysis indicated that at least six rats in each group would be required to achieve 80% power, with a significant P-value of 0.05 and a Cohen effect (f) of 0.6. Statistical analysis of the study was conducted using GraphPad software (Graph Pad Prism, San Diego, CA, USA). The mean values of the groups were evaluated with the One-Way ANOVA test. Values were reported as mean ± standard deviation (SD) and standard error of the mean (SEM). Post-hoc analysis was performed using Tukey's multiple comparison test, with statistical significance accepted at $p<0.05$.[7]

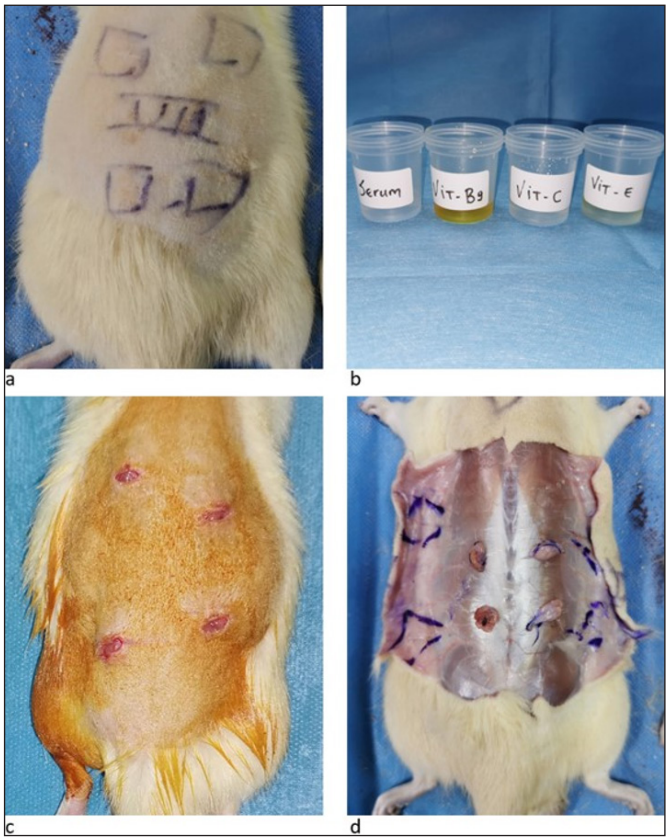

Figure 1: Visual representation of the surgical procedure (subfigures; a: four distinct quadrants in the dorsal region of the rats, b: preparation during incubation, c: placement of grafts, d: harvesting of grafts).

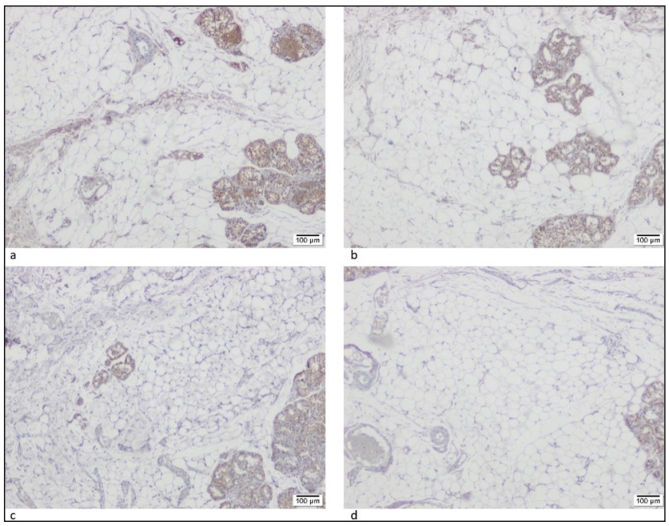

Figure 2: Perilipin staining for assessment (subfigures; a: control group, b: vitamin B9 group, c: vitamin C group, d: vitamin E group, scale 100 µm).

RESULTS

The results showed a statistically significant difference in the survival and integration of fat grafts among the vitamin B9, C, and E groups when compared with the control group ($p<0.001$ for each). While the vitamin E group exhibited a higher perilipin rate compared to other experimental groups (**Table 1**), these differences were considered statistically insignificant ($p>0.05$ for each) (**Table 2**).

Table 1. Results of Perilipin staining

Groups	N	Mean	SD	SEM
Control	10	0.08034	0.006592	0.002084
Vitamin B9	10	0.1176	0.01606	0.005077
Vitamin C	10	0.1197	0.02383	0.007536
Vitamin E	10	0.1332	0.01045	0.003303

Table 2. Statistical results in detail of two groups comparison

Groups Comparison	Difference	q value	p value
S vs B9	-0.03731	7.545	p<0.001
S vs C	-0.03935	7.958	p<0.001
S vs E	-0.05283	10.683	p<0.001
B9 vs C	-0.002042	0.4128	p>0.05
B9 vs E	-0.01552	3.138	p>0.05
C vs E	-0.01348	2.725	p>0.05

S: serum group, B9: vitamin B9 group, C: vitamin C group, E: vitamin E group, vs: versus

DISCUSSION

In recent decades, the utilization of autologous fat grafting procedures has experienced a remarkable proliferation within the domain of aesthetic and plastic surgery. This upsurge has, in turn, accentuated the importance of preserving the viability of transplanted fat, a matter of significant concern as these procedures continue to see extended application in clinical practice.[1] The endeavor to amplify the persistence and vitality of grafted fat has led to an exploration of the potential advantages associated with the incorporation of bioactive agents into fat grafting protocols.[2]

The affirmative outcomes observed in this study concerning vitamin B9 (Folic Acid) align with earlier research, underscoring its role in the stimulation of angiogenesis and collagen synthesis. These mechanisms could engender an environment conducive to graft vascularization and integration, thereby contributing to heightened graft survival.[2] Similarly, the antioxidative properties of Vitamin C (ascorbic acid), coupled with its participation in collagen synthesis, likely played a pivotal role in tissue repair, attenuating oxidative stress, and consequently supporting graft viability.[3] In a comparable vein, the pronounced antioxidative capacity of vitamin E (α-tocopherol) appeared to confer a protective shield against oxidative damage and inflammation, both recognized factors with the potential to curtail graft longevity.[4]

This present study embarked on the task of unraveling the potential benefits offered by folic acid, ascorbic acid, and α-tocopherol in enhancing the survival and viability of fat grafts. It's noteworthy that in our study, each rat was included in all experimental groups within a 'four quadrants' model.[5] This meticulous design served to minimize inter-rat variations in genetic, metabolic, and physiological factors. Consequently, the groups were rendered more homogenous, and the total number of experimental animals was kept at a minimum.

Through this rigorously devised rat model, the study scrutinized the impact of these vitamins on the aspects of fat graft integration, retention, and overall survival, thereby shedding light on their prospective roles as adjunctive agents to augment the efficacy of fat grafting procedures. The study findings revealed that the supplementation of vitamin B9, vitamin C, and vitamin E significantly influenced the survival and viability of fat grafts, findings that align harmoniously with existing literature.[8,9] A recent study, for instance, alluded to improved fat graft retention stemming from an elevation in the total antioxidant capacity in rats supplemented with vitamin C and E.[10] Notably, our experimental groups, those treated with these vitamins, exhibited substantially larger proportions of living fat cells in comparison to the control group (**Figure 3**). These results conspicuously allude to the favorable impact of vitamin B9, Vitamin C, and Vitamin E on the enhancement of graft integration, reduction of resorption, and an overall boost in viability. Collectively, these outcomes lend credence to the proposition that the incorporation of these vitamins can serve as valuable adjuncts in fortifying the effectiveness of fat grafting procedures, with the potential to elevate clinical outcomes within the realms of plastic and reconstructive surgery. It's paramount to emphasize, however, that while this study showcases promising results, the need for further research is unmistakable. This imperative is rooted in the necessity to fully elucidate the precise mechanisms underpinning the observed effects and to optimize the dosage and administration protocols of these vitamins within the context of fat grafting procedures.

In summation, this study provides robust evidence that vitamin B9, vitamin C, and vitamin E confer a constructive impact on the survival and viability of fat grafts. The implications of these observations have the potential to revolutionize fat grafting techniques, by presenting surgeons with additional tools to bolster graft retention and

to amplify overall procedural success. As the field of plastic and reconstructive surgery continues its evolution,[11-13] the integration of vitamin supplementation introduces an exciting avenue to enhance the reliability and durability of fat grafting procedures.[14] This, in turn, serves to benefit both the aesthetic and reconstructive aspects of patient care.[15-18]

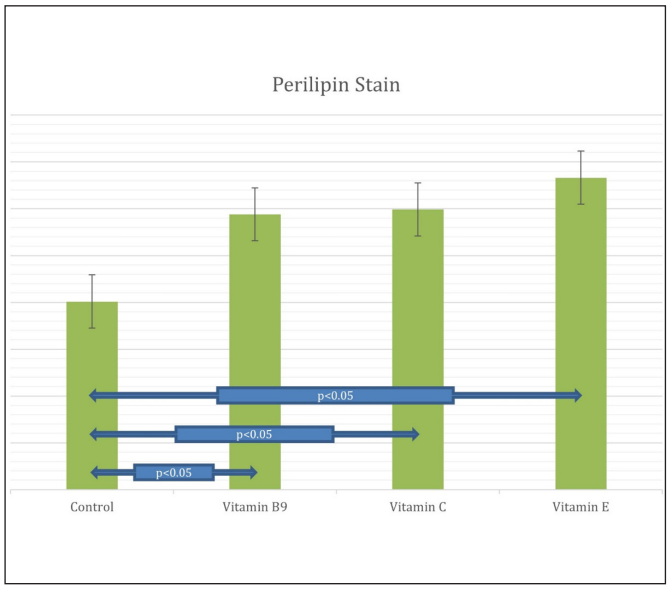

Figure 3: Comparative analysis of Perilipin staining rates.

Limitations

It's crucial to recognize that this study, being experimental in nature, inherits the inherent limitations associated with animal-based investigations. Furthermore, the study's reliance on a rat model necessitates caution when extrapolating these findings to human clinical scenarios.[19,20] The implantation model used in this study, while offering insights, may diverge from the cannula injection model more typical of clinical practice.

CONCLUSION

To summarize, the inquiry into the effects of vitamin B9 (folic acid), vitamin C, and vitamin E on fat graft survival has yielded promising findings. The study has compellingly demonstrated that these vitamins can exert a positive influence on graft viability, integration, and retention within a rat model. However, it remains imperative to delve deeper into factors such as dosage, administration methodologies, and the potential synergistic effects that might arise from the concurrent use of these vitamins. Future investigations could further explore the molecular pathways influenced by these vitamins, potentially unraveling the intricate interplay between angiogenesis, collagen synthesis, oxidative stress, and graft survival.

The notable enhancements in fat graft survival observed here underscore the potential value of these vitamins as valuable adjuncts for fortifying fat grafting procedures. Nevertheless, it is abundantly clear that further research is necessary to fully comprehend the underlying mechanisms, optimize dosages, and validate these findings in clinical settings. In this regard, clinical trials involving human subjects are indispensable to affirm the applicability of vitamin supplementation in the context of clinical fat grafting procedures. The findings of this study contribute to the expanding body of knowledge within the realm of plastic and reconstructive surgery, signifying a promising pathway toward improving the outcomes of fat grafting techniques and advancing patient care.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hatay Mustafa Kemal University Animal Experimental Researches Ethics Committee (Date: 21.09.2020, Decision No: 2020/06-6).

Informed Consent: Since this study was an animal experiment conducted in a laboratory, informed consent was not obtained.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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